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Evaluation of hepato and renal protective effect of synthesized nanoparticles using *Tinospora cordifolia* leaf extract

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ABSTRACT

In our current work, we have focused on AgNPs biosynthesis from the ethanolic leaf extract of *Tinospora cordifolia* and evaluated for their antioxidant, hepato and nephro-protective effects. The results depicted significant restoration in the serum levels of urea, creatine, and uric acid, SGOT and SGPT in potassium bromate induced Swiss albino mice to its normalcy upon treatment with AgNPs. The histopathological study on the kidney tissues correlates well with our biochemical observations. The overall results revealed that the synthesized AgNPs possess strong antioxidant, hepato and nephro-protectivity and directed towards the clinical use as a therapeutic agent.

1. Introduction

Several physical and chemical methods are commonly used for synthesizing nanoparticles[1]. Although silver ion reduction by chemical process is very effective, the toxic chemicals used in the method limits their use, and it also causes environmental contamination and health problems[2]. To avoid chemical toxicity, biological synthesis of nanoparticles from plants, enzymes, fungus and microorganisms were developed, and it is considered as an eco-friendly, non-toxic, biocompatibility, cost-effective as alternative to chemical and physical methods [3]. Potassium bromate ($KBrO_3$) is widely used as a food additive in food processor, beer making and also used in pharmaceutical and cosmetic industries[4]. When these products are used or consumed by humans, it causes cell lysis, generates free radicals, induces oxidative stress to cellular components, leading to renal failure, carcinogenesis and other organ damages[5]. Hence, in this study we have used $KBrO_3$ as a renal toxin to analyze the efficacy of the synthesized nanoparticles (AgNPs). One of the prime medical plant species of *Menispermaceae* family is *T. cordifolia*, which exhibits wide spectrum of pharmacological activities and also possesses anticancer, antiperiodic, antileprotic, anti-HIV, anti-inflammatory, anti-spasmodic, antiallergic, phagocytic activation, macrophages killing and antidiabetic properties[6]. The synthesis of AgNPs from the ethanolic extract of *T. cordifolia* has been reported earlier, but the nephro-protective effects of the AgNPs are not reported so far.

2. Materials and methods

2.1. *T. cordifolia* leaf extract preparation and silver nanoparticles biosynthesis

Fresh leaves of *T. cordifolia* were collected from Pudhuwayal, near Karaikudi, Sivagangai District, Tamilnadu. 50 g of *T. cordifolia* powder was subjected to ethanolic extraction (500 ml) and the sample was air dried and stored at 4 °C. 10 g of *T. cordifolia* powder was topped with 50 ml of 1mM aqueous silver nitrate ($AgNO_3$) solution. The change of colour of the reaction mixture during the incubation period depicts the reduction of AgNPs. The biosynthesized AgNPs solution was subjected to centrifugation at 10,000 rpm (4 °C), the obtained pellet was washed in sterile deionized distilled water and freeze dried in a lyophilizer.

2.2. Bio-physical characterization of AgNPs

The biological reduction of silver (Ag^+) ion in aqueous phase was

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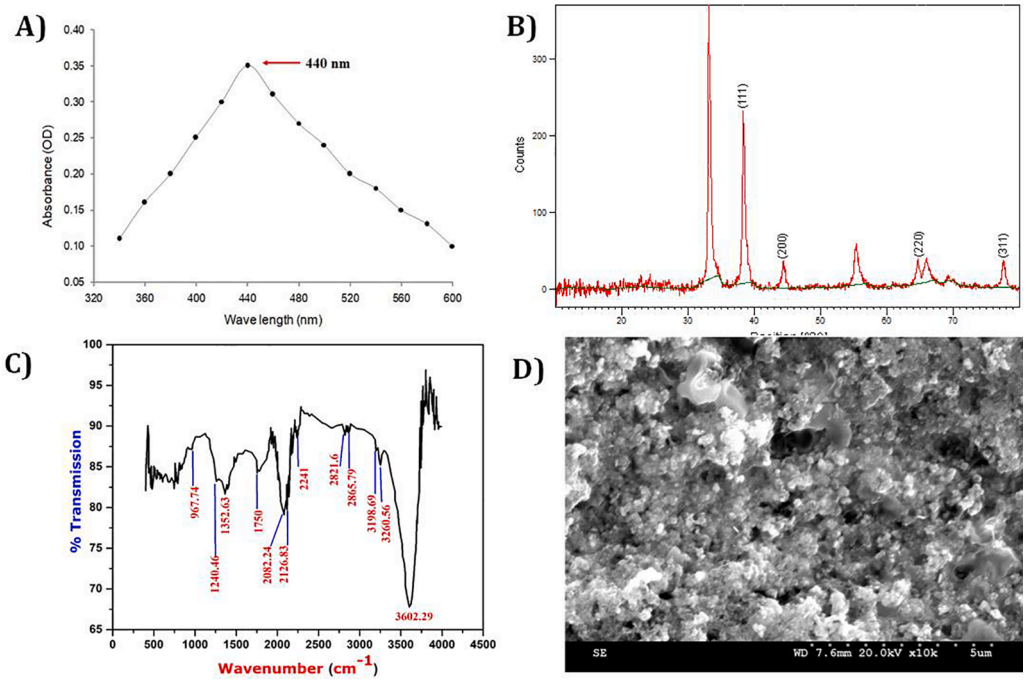

Fig. 1. A) UV-Vis absorption, **B)** XRD, **C)** FTIR, **D)** SEM analysis of AgNPs synthesized using *T. cordifolia* leaf extract.

monitored by sampling (0.3 ml) the suspension under Shimadzu UV-1800 spectrophotometer at different wavelength at 1 nm resolution. Thermo scientific Nicolet iS50 FTIR Spectrometer was used for the chemical bond analysis of the molecules in a solution of 4 cm^{-1}. Further, X-ray diffraction (XRD) analysis using Malvern Panalytical X'pert PRO was carried for the surface coated AgNPs on the grid to record the spectra at 40 kV and 30 mA with Cu-Kα radiation. Average crystalline size of the AgNPs synthesized through the process was calculated using Debye Sherrer's equation ($D = 0.94\lambda/\beta COS\theta$). The size, shape and morphology of the AgNPs was characterized using Hitachi S-4500 SEM.

2.3. In vivo studies

2.3.1. Experimental animal

Swiss albino mice (Balb/c) in the weight around 20 to 30 g were selected for the present study. Animals were handled as per the existing guidelines of the ethical committee for the handling of experimental animals, New Delhi. Ethical clearance was also obtained from the committee for animal usage (Reg.no.1086/AC/07/CPCSEA).

2.3.2. Experimental design

The randomly selected laboratory grown animals were divided into 5 groups and three mice comprising each group. The experimental duration was 14 days.

Group-I:Control mice
Group-II:10 ppm of $KBrO_3$ (10 mg/kg/day)
Group-III:Intoxicated mice treated with *T. cordifolia*
Group-IV:Intoxicated mice treated with AgNPs
Group-V:Vitamin E (100 mg/kg/day)

At the end of the experimental period, the mice were overnight deprived of food and then anesthetized with chloroform before being sacrificed. The constitutes are centrifuged for 10 min at 2500 g to separate the serum, and used for biochemical analysis. The tissue samples were processed and examined under light microscope to observe renal histopathological and ultrastructural changes.

2.3.3. Biochemical analysis

Biochemical analysis was carried out in serum samples of both control and experimental animals of each group. Level of SGOT and SGPT (Liver function test), total protein, Uric acid, Creatinine, Urea were assessed[7].

2.4. In vitro antioxidant assays

Sindhu *et al.* (2014), described methods were implemented to estimate the AgNPs ability in 1,1-Diphenyl-2-picrul-hydrazil (DPPH) and H_2O_2 scavenging[8].

3. Results and discussion

Previous report illustrates the AgNPs synthesized from *T. cordifolia* showed photocatalytic, antibacterial, pediculocidal and larvicidal activities[9,10]. Here, we intend to evaluate the hepato and nephroprotective activity of AgNPs synthesized form *T. cordifolia*.

3.1. Synthesis and characterization of AgNPs

The absorbance spectrum of AgNPs synthesized using the leaf extracts of *T. cordifolia* was estimated at different wavelengths ranging from 320 to 600 nm in UV-Vis spectroscopy. A strong absorbance peak at 440 nm evidenced the synthesize of nanoparticle[11] (Fig. 1A). XRD spectrum of AgNPs confirmed the presence of face-centered cubic nanocrystals as proved by the Braggs reflection peaks at 44.48°, 38.45°, 64.69°, and 77.62° which corresponds to (200), (111), (220), and (311) lattice planes indicate the formation of pure silver nanoparticles[12] (Fig. 1B). The synthesized AgNPs correspond to the structural arrangement of face centered cubic and the Debye Scherrers equation estimates the average size of the nanoparticle is 31 nm. The FTIR results showed that the synthesized AgNPs expressed visible bands at 3602, 3260, 3198, 2865, 2821, 2241, 2126, 2082, 1750, 1352, 1240, 1628, 1098 and 967 cm^{-1} (Fig. 1C). The band seen at 3602, 2865, 2921, 2126, 2082, 1750, 1628, 1352 and 1240 are attributed to hydrogen bonded OH species, CH_3 group, $(CH_3)_2N$ absorption, carbon-carbon triple bond stretch, carbonyl stretch C═O, C—H stretching vibrations and C-N stretching of amines, respectively. The SEM results depicted that the synthesized AgNPs are in cubic shape with the size ranging from 15 to 50 nm and are

Table 1
Effect of AgNPs on serum biochemical parameters in control and experimental animals (values are mean ± SD).

Hepatic parameters				
Groups	Treatments	SGOT (IU/L)	SGPT (IU/L)	Serum protein level (mg/100gm)
I	Control	210.31 ± 0.64^{c}	265.93 ± 0.92^{b}	67.15 ± 0.24^{d}
II	Potassium bromate	328.02 ± 0.07^{b}	374.56 ± 0.72^{a}	71.89 ± 1.35^{c}
III	Potassium bromate + EETC	262.31 ± 0.43^{a}	302.18 ± 1.34^{c}	69.21 ± 0.23^{b}
IV	Potassium bromate + AgNPs	231.56 ± 0.17^{e}	280.28 ± 0.62^{d}	73.58 ± 0.65^{a}
V	Potassium bromate + Vitamin-E	240.22 ± 0.38^{d}	292.14 ± 0.86^{e}	64.32 ± 0.45^{d}
Renal Parameters				
Groups	Treatments	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
I	Control	30.55 ± 1.89^{a}	12.58 ± 0.30^{c}	6.90 ± 0.20^{b}
II	Potassium bromate	58.78 ± 3.24^{b}	24.66 ± 0.46^{a}	14.70 ± 0.40^{c}
III	Potassium bromate + EETC	36.42 ± 1.43^{c}	15.72 ± 0.39^{b}	9.20 ± 0.70^{a}
IV	Potassium bromate + AgNPs	41.35 ± 2.05^{d}	17.32 ± 0.41^{c}	7.80 ± 0.30^{b}
V	Potassium bromate + Vitamin-E	40.73 ± 2.16^{e}	18.38 ± 0.39^{d}	10.40 ± 0.80^{e}

One-way ANOVA *P* values are significantly different at a < 0.001; b < 0.01; c < 0.005.

partially aggregated (Fig. 1D).

3.2. *In vivo studies*

3.2.1. *Effect of T. Cordifolia capped AgNPs on the serum SGOT and SGPT level of liver damaged swiss albino mice*

In the present experiments, a significant increase in the SGOT and SGPT level was observed in Group-II (intoxicated) on comparison with control mice in Group-I (Table 1), indicated hepatic damage. However, significant drop in SGOT and SGPT levels were observed by the treatment with *T. cordifolia* extract and *T. cordifolia* capped AgNPs denoting hepatocytes regeneration (Group-III and IV). Notably, overall viable rate was observed among the treatment groups (*T. cordifolia* extract and *T. cordifolia* capped AgNPs).

3.2.2. *Effect of T. Cordifolia capped AgNPs on the urea, creatine, and uric acid of renal damaged swiss albino mice*

The intoxicated mice (Group-II) have shown a higher level of creatine, urea, and uric acid as compared with Group-I (Table 1). The increased level of creatinine and urea are significantly linked with several medical conditions such as nephritis, nephron damage, renal ischemia, certain other renal diseases and urinary tract obstruction. Group-IV (Intoxicated mice by the treatment with AgNPs) have shown the decreased level of urea, creatine and uric acid significantly which is similar to the result of leaf extract (Group-III) and vitamin E (Group-V). However, after the treatment with *T. cordifolia* AgNPs (Group-IV), level of serum transaminases reduced to normal levels denotes the capability of AgNPs in the nephrocytes regeneration and healing effect on the renal parenchyma.

3.2.3. *Histopathological studies*

Histopathological sections of the kidneys of normal animals showed normal glomeruli and tubules (Fig. 2A). In the potassium bromate-intoxicated group, functions of the kidney were disrupted such as degenerated tubules with replaced fibrosis (Fig. 2B) whereas, *T. cordifolia* extract and nanoparticles-treated groups have shown normal function of the kidney (Fig. 2C and Fig. 2D). Further, the

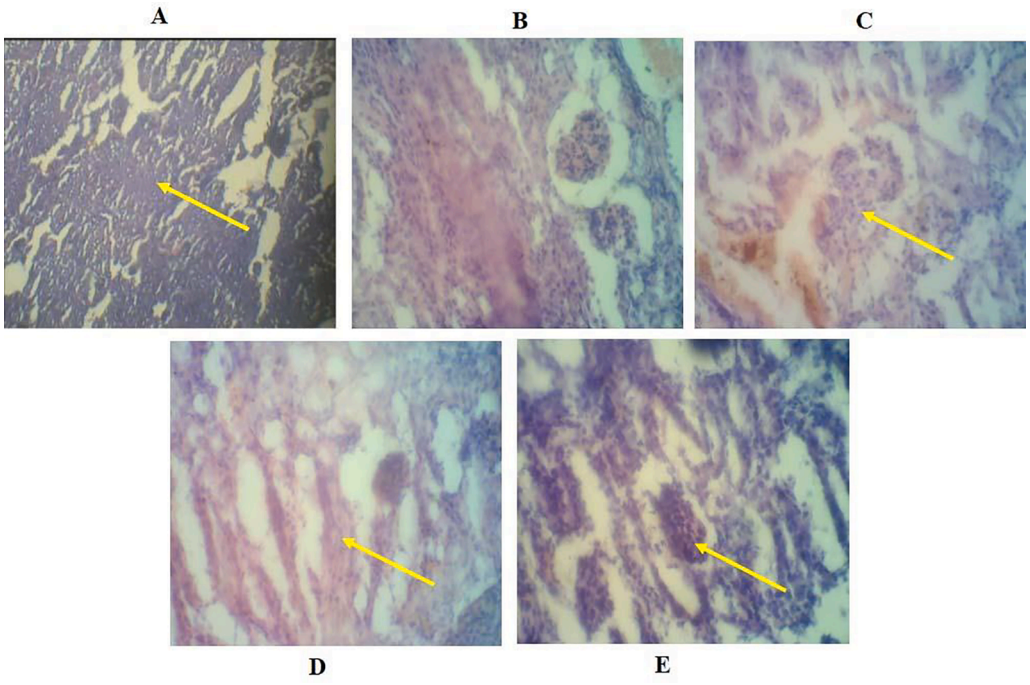

Fig. 2. Histopathology of kidney; **(A)** control; **(B)** intraperitoneally injected with $KBrO_3$; **(C)** mice treated with ethanolic extract of *T. cordifolia*; **(D)** mice with AgNPs and **(E)** mice with Vitamin-E. Arrow indicates kidney cells with normal glomeruli and tubules.

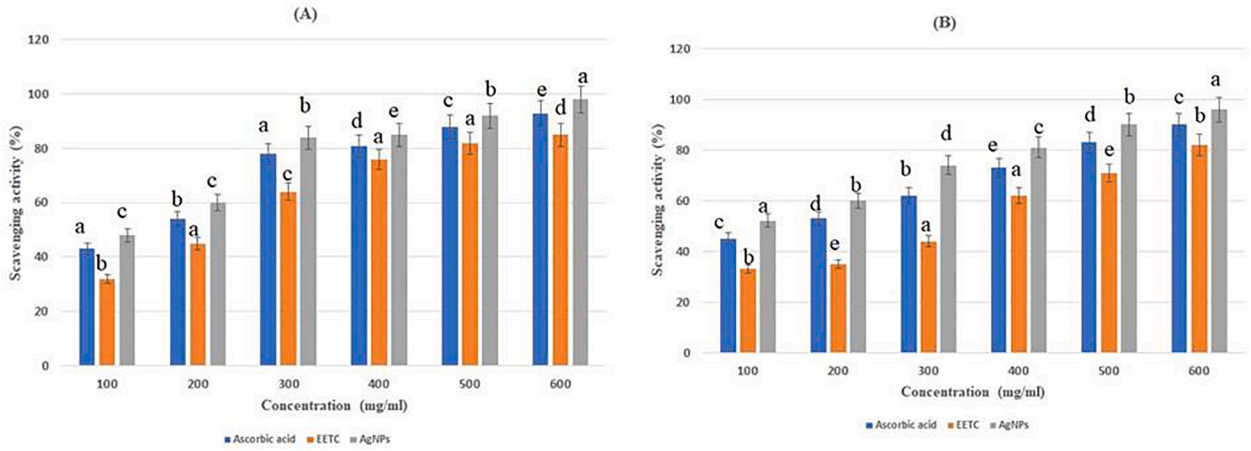

Fig. 3. *In vitro* antioxidant activity. **(A)** DPPH free radical scavenging activity, **(B)** Hydrogen peroxide scavenging activity.

intoxicated group treated with vitamin-E showed normal observation of kidney cells[13] (Fig. 2E).

3.3. In vitro antioxidant activity

3.3.1. DPPH radical scavenging activity

A significant increase in DPPH radical scavenging activity was noticed following treatment with AgNPs as compared to the standard ascorbic acid (Fig. 3A). The percentage of DPPH radical scavenging activity of AgNPs at 100 and 600 mg/ml was 48%. Likely, the standard molecule ascorbic acid showed 43% at 100 mg/ml and 93% at 600 mg/ml. The ethanolic extract of *T. cordifolia* exhibited 85% DPPH scavenging activity at 600 mg/ml.

3.3.2. Hydrogen peroxide scavenging activity

The ethanolic extract of *T. cordifolia* showed 33% and 82% scavenging activity at 100 and 600 mg/ml respectively. Interestingly, H_2O_2 radical scavenging activity was prominently increased upon treatment with *T. cordifolia* capped AgNPs compared to the ethanolic extract and the standard ascorbic acid. At 100 and 600 mg/ml of AgNPs, the percentage scavenging activity was 52% and 96% respectively (Fig. 3B).

4. Conclusion

The AgNPs synthesized from the *T. cordifolia* ethanolic leaf extract reduced the level of SGOT, SGPT, urea, creatine and uric acid significantly, thus exhibiting anti-oxidant, hepato and renal protective activity. Therefore, the synthesized AgNPs can be considered as a valuable candidate for therapeutic applications in targeted drug delivery for liver and kidney diseases.

CRediT authorship contribution statement

Muniyandi Biruntha: Methodology, Validation, Writing – original draft, Writing – review & editing. **Balan Karunai Selvi:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **James Arockia John Paul:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Pethanan Sivakumar:** Validation, Formal analysis, Investigation, Data curation, Writing – original draft. **Sundararaj Rajamanikandan:** Data curation, Writing – original draft, Writing – review & editing. **Dhamodharan Prabhu:** Data curation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] S. Iravani, H. Korbekandi, S. V. Mirmohammadi, B. Zolfaghari. Res. Pharm. Sci. 9 (2014) 385./pmc/articles/PMC4326978/(accessed November 22, 2021).
[2] K. Gudikandula, S. Charya Maringanti, J. Exp. Nanosci. 11 (2016) 714–721, https://doi.org/10.1080/17458080.2016.1139196.
[3] S.V. Otari, R.M. Patil, N.H. Nadaf, S.J. Ghosh, S.H. Pawar, Mater. Lett. 72 (2012) 92–94, https://doi.org/10.1016/J.MATLET.2011.12.109.
[4] Y. Kurokawa, A. Maekawa, M. Takahashi, Y. Hayashi, Environ. Health Perspect. 87 (1990) 309, https://doi.org/10.1289/EHP.9087309.
[5] M.K. Ahmad, S. Amani, R. Mahmood, Environ. Toxicol. 29 (2014) 138–145, https://doi.org/10.1002/TOX.20780.
[6] S. Saha, S. Ghosh, Anc. Sci. Life. 31 (2012) 151, https://doi.org/10.4103/0257-7941.107344.
[7] S. Rajamanikandan, T. Sindhu, D. Durgapriya, D. Sophia, P. Ragavendran, V. K. Gopalakrishnan, Asian Pac. J, Trop. Med. 5 (2012) 862–867, https://doi.org/10.1016/S1995-7645(12)60161-8.
[8] T. Sindhu, S. Rajamanikandan, P. Srinivasan. Indian J. Pharm. Sci. 76 (2014) 170./pmc/articles/PMC4023288/.
[9] K. Selvam, C. Sudhakar, M. Govarthanan, P. Thiyagarajan, A. Sengottaiyan, B. Senthilkumar, T. Selvankumar, J. Radiat. Res. Appl. Sci. 10 (2017) 6–12, https://doi.org/10.1016/J.JRRAS.2016.02.005.
[10] C. Jayaseelan, A.A. Rahuman, G. Rajakumar, A. Vishnu Kirthi, T. Santhoshkumar, S. Marimuthu, A. Bagavan, C. Kamaraj, A.A. Zahir, G. Elango, Parasitol. Res. 109 (2011) 185–194, https://doi.org/10.1007/S00436-010-2242-Y/FIGURES/4.
[11] J.M. Ashraf, M.A. Ansari, H.M. Khan, M.A. Alzohairy, I. Choi. Sci. Reports 2016 61. 6 (2016) 1–10. https://doi.org/10.1038/srep20414.
[12] D. Sundeep, T. Vijaya Kumar, P.S.S. Rao, R.V.S.S.N. Ravikumar, A. Gopala Krishna. Prog. Biomater. 2017 61. 6 (2017) 57–66. https://doi.org/10.1007/S40204-017-0067-9.
[13] F. Alghamdi, M.N. Al-Seeni, M.A. Ghoneim, Clin. Nutr. Exp. 32 (2020) 29–37, https://doi.org/10.1016/J.YCLNEX.2020.05.002.